A MICROORGANISM PRODUCING 5'-XANTHYLIC ACID

[Technical Field]

The invention relates to a microorganism producing 5'-xanthylic acid. More particularly, the invention relates to a mutant strain of *Corynebacterium ammoniagenes* KCCM 10448, which is given a resistance to 5-fluorotryptophan, a metabolite analogue of tryptophan, in order to enhance purine biosynthesis, making it possible to enhance N_5, N_{10}-tetrahydrofolate involved in purine biosynthesis pathway, and to accumulate 5'-xanthylic acid in culture medium at a high yield and high concentration rate for same period of fermentation.

[Background Art]

5'-xanthylic acid is an intermediate in the nucleic acid biosynthesis process, which is physiologically important in the body of animals and plants, used in food, medical supplies and other various field. Thus, inventors of the invention developed a mutant strain having a resistance to 5-fluorotryptophan, from a known strain *Corynebacterium ammoniagenes* KCCM 10448 also developed by inventors of the invention, producing 5'-xanthylic acid at a high yield and high concentration rate by a direct fermentation method.

5'-xanthylic acid is an intermediary product of purine nucleotide biosynthesis process and important material for producing 5'-guanylic acid. A widely used method to produce 5'-guanylic acid having fineness and high quality is microorganism fermentation method which produces 5'-xanthylic acid first and converts it into 5'-guanylic acid enzymatically, therefore, to produce 5'-guanylic acid, corresponding amount of 5'-xanthylic acid is necessary. Conventional methods to produce 5'-xanthylic acid are chemosynthesis, deaminization of 5'-guanylic acid which is produced as a result of decomposition of ribonucleic acid

in yeast, a fermentation method to add xanthine as precursor material in fermenting medium, a fermentation method to use a mutant strain of microorganism, a method to add antibiotic material (JP 1477/42 and JP 20390/44), a method to add surfactant (JP 3825/42 and JP 3838/42) and so on. Among these, a direct fermentation method of 5'-xanthylic acid by a mutant strain of microorganism is quite advantageous in terms of industrial aspect. Thus, we inventors developed a mutant strain with increased productivity of 5'-xanthylic acid, by modifying the existing character of *Corynebacterium ammoniagenes* KCCM 10448 into the character of producing 5'-xanthylic acid at a large yield rate.

[Disclosure of the Invention]

[Technical Problem]

The biosynthesis pathway for producing XMP is very complicated, and the reaction in which various amino acids and coenzymes participate continues. Especially, N_5, N_{10}-tetrahydrofolate participates in two steps among six steps reactions from PRPP(Phosphoribosylpyrophosphate) to XMP, and plays a role in transferring formyl group to each precursor. Meanwhile, p-aminobenzoate, synthesized from chorismate, is necessary for biosynthesis of N_5,N_{10}-tetrahydrofolate. Chorismate is an intermediary product in tryptophan biosynthesis process and the inventors thought that enhancement of producing chorismate results in enhancement of producing N_5, N_{10}-tetrahydrofolate.

Thus, the inventors examined a mutant strain, which is given a resistance to 5-fluorotryptophan, a metabolite analogue of tryptophan, in order to enhance purine biosynthesis, and which enhances biosynthesis of chorismate and increases N_5,N_{10}-tetrahydrofolate, and found out that a mutant strain having a resistance to 5-fluorotryptophan is very effective and can produce 5'-xanthylic acid at a high

yield and high concentration rate by a direct fermentation method than prior art, and accomplished in this invention.

[Technical Solution]

Now, a method for separating and taking the microorganism of the present invention is explained in detail.

The microorganism of the invention, *Corynebacterium ammoniagenes* CJXFT 0301 (KCCM-10530) is obtained by treating *Corynebacterium ammoniagenes* KCCM 10448 as parent strain with UV radiation and mutation derivatives such as N-methy-N'-nitro-n-nitrosoguanidine(NTG) according to ordinary procedure, and selecting a mutant strain among these which can grow in the culture medium (glucose 20g/L, potassium phosphate monobasic 1g/L, potassium phosphate dibasic 1g/L, urea 2g/L, ammonium sulfate 3g/L, magnesium sulfate 1g/L, calcium chloride 100mg/L, ferrous sulfate 20mg/L, manganese sulfate 10mg/L, zinc sulfate 10mg/L, biotin 30μg/L, thiamine hydrochloride 0.1mg/L, copper sulfate 0.8mg/L, adenine 20mg/L, guanine 20mg/L, pH 7.2) which different concentration levels of fluorotryptophan (10,20,50,70,100,200mg/L) is added into. In the procedure, 0~200mg/L fluorotryptophan was added into the medium. Parent strain showed a resistance up to 20mg/L fluorotryptophan but no growth was observed at the concentration level above 50mg/L, therefore the inventors separated a strain which can grow in 100mg/L fluorotryptophan, named CJXFT 0301, and deposited it under Budapest Treaty to the Korean Culture Center of Microorganisms on November 25, 2003 with accession Number KCCM 10530.

The biochemical characteristic of the novel mutant strain CJXFT 0301 of the invention is shown in the following Table 1. According to the Table 1, the microorganism of the invention can grow in the medium which 100mg/L

fluorotryptophan was added into.

Table 1

Strain	Fluorotryptophan Concentraion (mg/L)						
	0	10	20	50	70	100	200
KCCM 10448	+++	++	+	-	+	-	-
CJXFT 0301	+++	+++	+++	+++	++	+	-

The medium was fermented at 30℃ for 5 days.

+ : growth, - : no growth

[Advantageous Effects]

The invention adopted *Corynebacterium ammoniagenes* KCCM 10448 as parent strain and treated it UV radiation or mutation derivatives such as N-methy-N'-nitro-n-nitrosoguanidine (NTG) according to ordinary procedure, to obtain a mutant strain. The mutant strain is given a resistance to 5-fluorotryptophan, in order to enhance biosynthesis of N_5,N_{10}-tetrahydrofolate used for transferring two formyl group during the process of purine biosysthesis, and the strain has an effect on accumulating 5'-xanthylic acid in culture medium at a high yield and high concentration rate for same period of fermentation.

[Mode for Carrying Out the Invention]

Example 1

Used strains: *Corynebacterium ammoniagenes* KCCM 10448, *Corynebacterium ammoniagenes* CJXFT 0301 (KCCM 10530)

Seed medium: glucose 30g/L, peptone 15g/L, yeast extract 15g/L, sodium chloride 2.5g/L, urea 3g/L, adenine 150mg/L, guanine 150mg/L, pH 7.2

Fermentation medium: (1) A medium: glucose 60g/L, magnesium sulfate 10g/L, ferrous sulfate 20mg/L, zinc sulfate 10mg/L, manganese sulfate 10mg/L, adenine 30mg/L, guanine 30mg/L, biotin 100μg/L, copper sulfate 1mg/L, thiamine hydrochloride 5mg/L, calcium chloride 10mg/L, pH 7.2

(2) B medium: potassium phosphate monobasic 10g/L, potassium phosphate dibasic 10g/L, urea 7g/L, ammonium sulfate 5g/L

Fermentation method: 5mL of the seed medium was poured into a test tube having diameter of 18mm and sterilized under pressure according to the common methods. After the sterilization, *Corynebacterium ammoniagenes* KCCM 10448 and *Corynebacterium ammoniagenes* CJXFT 0301 were seeded into respectively and it was cultured with shaking at 180rpm, 30℃ for 18 hours. The resultant was used as seed culture. Then, as fermentation medium, A medium and B medium were sterilized separately under pressure according to the common methods and 29mL of A medium and 10mL of B medium were respectively poured into sterilized 500mL-Erlenmeyer flask for shaking and 1mL of the above-mentioned seed culture was seeded into and fermented at 200rpm, 30℃ for 90 hours. After the fermentation was completed, the amount of accumulation of 5'-xanthylic acid in the medium showed that the amount in KCCM 10448 was 25.4g/L and the amount in CJXFT 0301 was 28.6g/L. (The concentration of accumulated 5'-xanthylic acid is given by 5'-sodium xanthate·$7H_2O$.)

Example 2

Used strains: same as example 1.

Primary seed medium: same as the seed medium of example 1.

Secondary seed medium: glucose 60g/L, potassium phosphate monobasic 2g/L, potassium phosphate dibasic 2g/L, magnesium sulfate 1g/L, ferrous sulfate 22mg/L, zinc sulfate 15mg/L, manganese sulfate 10mg/L, copper sulfate 1mg/L, calcium chloride 100mg/L, biotin 150μg/L, adenine 150mg/L, guanine 150mg/L, thiamine hydrochloride 5mg/L, antifoaming agent 0.6mL/L, pH 7.2

Fermentation medium: glucose 151g/L, phosphoric acid 32g/L, potassium hydroxide 25g/L, adenine 198mg/L, guanine 119mg/L, ferrous sulfate 60mg/L, zinc sulfate 42mg/L, manganese sulfate 15mg/L, copper sulfate 2.4mg/L, alaniate 22mg/L, NCA 7.5mg/L, biotin 0.4mg/L, magnesium sulfate 15g/L, cystinate 30mg/L, histidinate 30mg/L, calcium chloride 149mg/L, thiamine hydrochloride 15mg/L, antifoaming agent 0.7mL/L, CSL 27mL/L, tuna extract 6g/L, pH 7.3

Primary seed culture: 50mL of the primary seed medium was poured into 500mL-Erlenmeyer flask for shaking and sterilized under pressure at 121℃ for 20 minutes. After cooling, *Corynebacterium ammoniagenes* KCCM 10448 and *Corynebacterium ammoniagenes* CJXFT 0301 were seeded into respectively and it was cultured with shaking at 180rpm, 30℃ for 24 hours.

Secondary seed culture: The secondary seed medium was poured into 5L-experimental fermentation baths (2L each) and sterilized under pressure at 121℃ for 20 minutes. After cooling, 50mL of the above primary seed culture was seeded and cultured with the air supply of 0.5vvm, at 900rpm, 31℃, for 24 hours. During the culturing process, the pH level of the medium was kept at 7.3 with adjusting

by ammonia solution.

Fermentation method: The fermentation medium was poured into 30L-experimental fermentation baths (8L each) and sterilized under pressure at 121℃ for 20 minutes. After cooling, the above secondary seed culture was seeded into (1.5L each) and cultured with the air supply of 1vvm, at 400rpm, 33℃. Whenever the residual sugar level drops below 1% during the culturing process, sterilized glucose was supplied and the total sugar level in the fermentation medium was kept at 30%. During the culturing process, the pH level of the medium was kept at 7.3 with adjusting by ammonia solution and the process took 80 hours. After the fermentation was completed, the amount of accumulation of 5'-xanthylic acid in the medium showed that the amount in KCCM 10448 was 145.2g/L and the amount in CJXFT 0301 was 155.4g/L. (The concentration of accumulated 5'-xanthylic acid is given by 5'-sodium xanthate·$7H_2O$.)

Applicant's or agent's file reference	International application No.
YL04023PCT	PCT/KR2004/002993

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 3, line 23-24.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

Korean Culture Center of Microorganisms

Address of depositary institution *(including postal code and country)*

Name: Korean Culture Center of Microorganisms

Address: 361-221, Yurim B/D, Hongje 1-dong, Seodaemun-gu
Seoul 120-091, Republic of Korea

Date of deposit	Accession Number
November 25, 2003	KCCM-10530

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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